Exhibit A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s) : PAWELEK, et al.

U.S. Serial No.: 10/723,570, a continuation of U.S. Serial No. 09/358,052, filed July 21, 1999, now U.S. Patent No. 6,685,935, issued February 3, 2004, which is a continuation of U.S. Serial No. 08/658,034, filed June 4, 1996, now U.S. Patent No. 6,190,657, issued February 20, 2001, which is a continuation-in-part of U.S. Serial No. 08/486,422, filed June 7, 1995, now abandoned

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For : VECTORS FOR THE DIAGNOSIS AND TREATMENT OF SOLID TUMORS INCLUDING MELANOMA

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Sir/Madam:

AFFIDAVIT UNDER 37 CFR 1.132

My name is David Bermudes. I have BA in Biology from Oberlin College and a Ph.D. in Cell and Molecular Biology from Boston University. I am currently employed at Vion Pharmaceuticals as Director of Microbiology. I have 12 years of experience in the field of microbial pathogenesis and genetic engineering of bacteria.

I wish to affirm to the ability of one ordinarily skilled in the arts to construct an *E. coli* strain according to the invention for inhibiting the growth of a solid tumor cancer, comprising administering to a patient having a solid tumor, a tumor specific *Escherichia coli* genetically engineered to express a suicide gene. The embodiments we described using *Salmonella*, although novel and non-obvious, was merely exemplary and, thus, extrapolation to use *E. coli* would not require *undue* experimentation for one of ordinary skill in the art due to the known biological similarities of *E. coli* with *Salmonella*. The strong similarities were noted Riley and Krawiec in 1987 Chapter 56. Genome Organization., pp 967-981, In: Escherichia coli and Salmonella typhimurium, Cellular and Molecular Biology Neidhardt et al., eds, 1987); "By aligning the genetic maps of *E. coli* and *S. typhimurium*, one sees first of all the order of genes on the two maps is nearly identical..." It was further known that there was a high degree of DNA sequence homology, such as the gene *aroF*, which shares 85% nucleic acid homology and 96% amino acid homology between E. coli and Salmonella (Muday GK, Herrmann KM.Regulation of the *Salmonella typhimurium aroF* gene in *Escherichia coli*. *J Bacteriol*. 1990 May;172(5):2259-66.). Furthermore, in the same study, the *trp* repressor element recognized the regulatory elements equally between both species, further

indicating the level of similarity and interchangeability of components between the two species. Therefore, one ordinarily skilled in the arts would assume that the methods would be interchangeable, even given minor chromosomal organizational differences or DNA sequence differences, and would obtain substantially the same results.

In regard to generation of superinfective *E. coli* strains, it was well-known at the time that *E. coli* was capable of invading mammalian cells (Epithelial cell invasion and adherence directed by the enterotoxigenic *Escherichia coli* tib locus is associated with a 104-kilodalton outer membrane protein. Elsinghorst EA, Weitz JA. Infect Immun. 1994 Aug;62(8):3463-71.). Although many *E. coli* strains invade mammalian cells less well than *Salmonella*, one ordinarily skilled in the arts would be capable of taking an *E. coli* strain and selecting for superinfection using the methods provided in the instant application because the mutation methods are the same for both species and the selection methods using gentimycin are applicable to both species (Elsinghorst, 1994 Methods in enzymology 236: 405-420; see first full paragraph on p 406). Although the amount of work to be performed is substantial, it would not require undue experimentation.

With regard to the ability to invade tumor cells, the Elsinghorst et al.1994 paper utilized HCT 8, a human colon tumor adenocarcinoma. Therefore, earlier studies established the ability to invade tumor cells, but not for inhibiting the growth of a solid tumor cancer, comprising administering to a patient having a solid tumor, a tumor specific *Escherichia coli* genetically engineered to express a suicide gene.

In regard to the ability to attenuate *E. coli*, the methods to mutate and select auxotrophic mutants were well known at the time. For example, the use of TN10 to generate uridine auxotrophs (Fonstein M, et al. Tn10-mediated inversions fuse uridine phosphorylase (udp) and rRNA genes of Escherichia coli. *J Bacteriol.* 1994 Apr;176(8):2265-71.). Furthermore, the similarity of *E. coli* and *Salmonella* was also well known at the time, and general comparison of the *E. coli* and *Salmonella* genetic maps shows that many of the genes share the same chromosomal organization (see description above). Therefore, application of the methods described in the instant application used to obtain attenuating auxotrophic mutations in *Salmonella* strains would be understood to produce and allow to be obtained, nearly identical mutations in *E. coli*.

In regard to the ability to obtain *E. coli* strains with altered lipid A molecules such those with the *firA* mutation, it was also was also well known at the time that the genes and their functions for lipid A biosynthesis were similar in *Salmonella* and *E. coli*. Roy et al., J. Bacteriol. 176: 1639-1646, 1994) remark in the first line of their introduction "The *firA* gene of *Escherichia coli* and the analogous gene in *Salmonella typhimurium* have been shown to be essential for growth".

In regard to the ability of E. coli to express the suicide genes, it was well known at the time to those ordinarily skilled in the arts that the expression plasmids such as those used in the experiments well known at the time of the application to bear the colE1 origin of replication capable of being carried in both *E. coli* and *Salmonella*. Therefore, it would be understood that the suicide gene expression plasmids described for *Salmonella* would be carried produce the same effects in *E. coli*. In regard to the constitutive, inducible or tumor cell specific promoter, the similarity of *E. coli* and *Salmonella* promoters was also well known at the time as described above. Therefore, it would be understood that the suicide gene expression plasmids described for Salmonella would produce similar gene regulation in *E. coli*.

Furthermore, Yu et al. (Nature Biotechnology 22: 313-320, 2004) successfully applied some of the same techniques to E. coli that were used on Salmonella in the present case. They used E.

coli DH5α, an attenuated auxotrophic strain, which consists of the genotype F-, ϕ80d*lacZ*_M15, Δ(*lacZYA-argF*)U169, *deoR*, *recA*1, *endA*1, *hsdR*17(rk-, mk+), *phoA*, *supE*44, λ-, *thi*-1, *gyrA*96, *relA*1 and were able to demonstrate highly specific tumor targeting.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Signature

January 6, 2005
Date

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